REMARKS/ARGUMENTS

Status of the claims

Claims 1-8, 10-13, 22, and 23 are pending and under examination. No amendments have been introduced in this response.

Rejection under 35 U.S.C. § 103

The Examiner has maintained the rejection of claims 1-8, 10-13, 22 and 23 as allegedly obvious over U.S. Patent No. 6,124,110 to Wöber *et al.*; U.S. Patent No. 5,625,036 to Hawkins *et al.*; Lawson *et al.*, *J. Biol. Chem.* 267(7): 4834-4843 (1992); Váradi *et al.*, *J. Thromb. Haemostasis* 1:2374-2380 (2003); U.S. Patent No. 5,952,198 to Chan; U.S. Patent No. 6,074,826 to Hogan *et al.*; U.S. Patent No. 6,576,422 to Weinstein *et al.*; U.S. Patent No. 6,756,019 to Dubrow *et al.*, U.S. Patent Publication No. 2002/0151582 to Dou *et al*; and p. B-77 of the CRC Handbook of Chemistry and Physics 51st ed., R.C. Weast, ed. Applicants traverse this rejection for reasons of record.

The Examiner makes the following remarks regarding the response and Rule 1.132 Declaration submitted with Applicants' response filed September 24, 2007. First, with regard to the Rule 1.132 Declaration by Dr. Peter Turecek, the Examiner notes that in the results shown in Table 1, no precipitate forms for the first sample. The Examiner further indicates that the concentrations of ZGGR-AMC and $CaCl_2$ that were involved in the experiments described in the Declaration were not provided, nor were temperatures at which the solutions were incubated. The Examiner therefore alleges that the Declaration provides insufficient information to compare the data with the prior art and support that the claims are nonobvious. Applicants disagree. Provided herewith is a second Declaration under 37 C.F.R. § 1.132 by Peter Turecek (Turecek II) that provides further evidence that one of skill would not have expected that organic solvent could be omitted from a buffer for reconstituting a lyophilized mixture as set forth in the claims and still reproducibly and reliably dissolve the mixture.

The various aspects of the Examiner's remarks set forth in the current Office Action will be addressed in turn.

Precipitation is likely to occur during reagent preparation

In the characterization of the teachings of Váradi *et al.* and Lawson *et al.* at page 3 of the Office Action, the Examiner interprets the concentration of ZGGR-AMC in Váradi *et al.* as being less than 0.5 mM and the concentration of $CaCl_2$ as being less than 15 mM. With regard to Lawson *et al.*, the Examiner characterizes the concentration of $CaCl_2$ as 5 mM and the concentration of the fluoresecent substrate as less than 1 mM. The Examiner notes that these references do not indicate that anything precipitates during any steps of the assay. The Examiner therefore considers the precipitate that forms, as noted in the specification and Declaration by Dr. Turecek, to be a "new result". Applicants respectfully disagree with this analysis.

The Turecek II Declaration provides additional clarification on this issue. The Declaration provides a more detailed description of experiments that were previously performed and described in his first Declaration. In Experiment 1, 25 mg of the fluorescent substrate ZGGR-AMC.HCl in powder form was dissolved in 7.4 ml of a buffer containing 10% DMSO. After stirring until the substrate was fully dissolved, 0.58 ml of 1 M $CaCl_2$ was added. Experiment 2 employed the similar amounts of reagents, but was also repeated in triplicate. In each of experiments 3 and 4, 250 mg of ZGGR-AMC was dissolved in 74 ml of buffer containing 10% DMSO. After the substrate was fully dissolved, 6 ml of 1 M $CaCl_2$ solution was added. As shown in sections 6 and 7 and the attachment, a precipitate occurred in five out of six individual samples when $CaCl_2$ was added to the fluorogenic thrombin substrate (see, also, section 8, which summarizes the results of the experiments). The fluorescent thrombin substrate had been dissolved, with stirring, at room temperature in a buffer containing 10% DMSO. The addition of $CaCl_2$ to substrate was performed at room temperature. As noted, a precipitate formed in almost all of the samples. Additional stirring at room temperature of 37°C was required to dissolve the precipitate. Thus, during the preparation of the reagents, the evidence shows that precipitation is in fact highly likely to occur upon the addition of the $CaCl_2$ to the thrombin substrate that had been dissolved in the DMSO-containing buffer.

The concentrations employed reflect those used in reagent preparation

Dr. Turecek also explains that although larger concentrations (relative to the concentration of fluorogenic thrombin substrate and $CaCl_2$ in the final substrate/calcium chloride solution employed by Váradi *et al.*) were used in the experiments presented in the Turecek II Declaration, these concentrations were employed because they are typical for generating a working solution of the substrate/calcium chloride. Dr. Turecek then explains that for the experiments described by Váradi *et al.*, which is his own work, the fluorogenic substrate also has to be dissolved in a buffer containing DMSO and that precipitation also occurred when the fluoregnic substrate and $CaCl_2$ were combined, even though it was not mentioned in the publication.

As explained in section 9 of the Turacek II Declaration, for the experiments in Váradi *et al.*, the fluorogenic ZGGR-AMC sbustrate was first dissolved in a HEPES-NaCl buffer that contained 10% DMSO. When $CaCl_2$ was added to the dissolved ZGGR-AMC substrate, precipitation occurred. Warming and vigorous shaking were required to dissolve the precipitate. The concentrations in this solution were 75 mM $CaCl_2$ and 5 mM ZGGR-AMC. Once the precipitate was dissolved, the solution was aliquoted and stored at -20°C. When the solution was used, it was diluted 5-fold with HEPES-NaCl buffer. The concentrations of ZGGR-AMC and $CaCl_2$ in the solution used for the reaction was therefore 15 mM $CaCl_2$ and 1 mM thrombin substrate. Thus, precipitation of the calcium chloride-fluorogenic substrate in fact occurred during preparation of the thrombin substrate/calcium chloride solution for the experiments described in Váradi *et al.*, even though the publication itself is silent on this issue.

Fluorogenic substrates are dissolved in organic solvents

On page 5 of the Office Action, the Examiner contends that one of ordinary skill in the art would have recognized that the thrombin substrate and $CaCl_2$ are both soluble in water. The Examiner further contends that Váradi *et al.* and Lawson *et al.* both disclose water soluble fluorogenic thrombin substrates in dry form. Again, Applicants disagree with this assessment.

As Dr Turecek further explains in section 10 of the Turecek II Declaration, fluorescent thrombin substrates are rarely water soluble. After lyophilization, an organic solvent such as DMSO is required to re-dissolve the substrate. Indeed, according to the manufacturer, the commercially available ZGGR-AMC substrate has to be dissolved in an organic solution. Lawson *et al.* also describes fluorogenic thrombin substrates. However, these were first dissolved in DMSO to a stock concentration of 10 mM (see, *e.g.,* Lawson *et al.*, column 2, second and third full paragraphs on page 48336).

As further evidence of the poor solubility of the fluorescent substrates, an experiment was performed to try to reconstitute ZGGR-AMC (250 mg) in water (74 ml) without DMSO. This experiment is presented in section 10 of the Declaration. The ZGGR-AMC powder could not be fully dissolved. The $CaCl_2$ was added even though the substrate was not fully dissolved. A fine precipitate was formed. This could not be solubilized, even after heating to 37°C and stirring for 60 minutes (Experiment 5 and Figure 3).

The Examiner cites Wöber *et al.* as allegedly supporting the position that the thrombin substrate employed in the present invention and calcium chloride are both soluble in water or buffer. Applicants acknowledge that the substrate of Wöber *et al.* is soluble in water (column 5, lines 20-22); however, this is a chromogenic thrombin substrate, not a fluorogenic thrombin substrate. As Dr. Turecek has explained, fluorogenic thrombin substrates have markedly different solubility properties.

Last, the Examiner contends that the evidence presented in the previous response is also insufficient because Applicants have not shown that lyophilizing calcium chloride and a fluorescently labeled thrombin substrate together, rather than separately, changes either composition or produces a different composition when the two compound are combined in an aqueous solution. However, the basis for this assertion is not clear. Applicants have shown that there are known problems with the solubility of fluorescent thrombin substrates in water or an aqueous buffer, and that this solubility problem extends to the preparation of the substrate/calcium chloride solution in combination. Applicants have also provided direct evidence that fluorogenic thrombin substrates have only limited solubility in aqueous solutions.

Dr. Turecek explains in section 11 of the Turecek II Declaration that a basic requirement for reagent kits is that the kit be "ready to use" and immediately available for use, if needed. Therefore, a reagent in which a precipitate is likely to form (either immediately or delayed) upon the addition of $CaCl_2$, to the substrate is not an acceptable diagnostic reagent. Dr. Turecek explains that it was an unexpected finding that the current kit would be "ready to use" when the lyophilized mixture comprising the fluorogenic substrate and calcium chloride is reconstituted in an aqueous buffer. The DMSO present in the initial buffer to solubilize the fluorescent thrombin substrate and still present when the calcium chloride is added is lost during lyophilization. Based on his experience in this field working with fluorogenic substrates, it would not be expected that the organic solvent could be omitted when reconstituting a lyophilized mixture as set forth in the claims and still reproducibly and reliably dissolve the mixture.

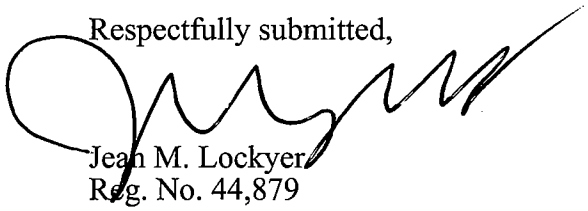
In considering obviousness, differences between the prior art and the invention as a whole must be considered (see, *e.g.,* MPEP § 2141.02). Here, the specification and Declarations presented by Dr. Turecek provide further evidence that one of skill would not have expected that a lyophilized mixture comprising $CaCl_2$ and a thrombin substrate comprising a fluorescent label would form a clear solution when dissolved in an aqueous solution. Accordingly, the claimed and methods using such kits are unobvious over the cited art. Applicants therefore respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Jean M. Lockyer
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
JML:jml
61287683 v1